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Original Article

Equine sarcoid: *in situ* demonstration of matrix metalloproteinase expression

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Abstract

Sarcoids are the most prevalent equine skin tumours and remain a therapeutic challenge due to their differing clinical morphology, local aggressive behaviour, and high recurrence following surgical treatment. *In vitro*, sarcoid derived fibroblasts are invasive and express matrix metalloproteinases (MMPs), namely MMP-1, -2 and -9.

We hypothesised that the MMPs produced by neoplastic cells play a role in both their local invasiveness and interaction with the overlying epidermis (picket fence formation). We therefore undertook a morphological study to investigate the local behaviour and *in situ* MMP expression pattern in sarcoids of different clinical types. A total of 43 surgically excised sarcoids were examined by histology, immunohistology for the expression of MMP-1, -2 and -9, and transmission electron microscopy.

Regardless of the clinical type, sarcoids showed local invasion of the dermis and damage to the basement membrane in areas of interaction with the epidermis. This was associated with MMP-1 expression in both neoplastic cells and epidermis. These results suggest a link between MMP-1 expression and the local aggressiveness of sarcoids regardless of the clinical type.

Keywords: Equine sarcoid; Matrix metalloproteinases; Dermal invasion; Picket fence formation; Basement membrane

58 **Introduction**

59 Sarcoids are the most common equine skin tumours worldwide. A prevalence between
60 1 and 8% (Knottenbelt, 2005) and a predilection for younger animals have been reported
61 (Torrontegui and Reid, 1994). Sarcoids develop after inoculation of fibroblasts with bovine
62 papillomavirus types 1 and 2 (BPV-1, -2) and viral transcription (Bogaert et al., 2007; Hartl
63 et al., 2011).

64 Sarcoids can typically be recognised clinically and histopathologically (Marti et al.,
65 1993; Knottenbelt, 2005). With inconclusive histopathological features, PCR from superficial
66 swabs, skin scrapings or the tumour mass can be helpful (Martens et al., 2000; Martens et al.,
67 2001a).

68 Six clinical types with individual growth characteristics and local behaviour are
69 described. These include occult, verrucose (‘warty’), nodular, fibroblastic, mixed and
70 malignant forms (Knottenbelt, 2005). Focal dermal accumulations of fibroblastoid cells,
71 usually in association with hyperplasia, hyperkeratosis and rete peg formation (RPF) of the
72 overlying epidermis are typical histological features. Neoplastic cells often show “picket
73 fence” alignment along the epidermis and are generally locally invasive (Marti et al., 1993;
74 Martens et al., 2000; Scott and Miller, 2003). The latter possibly accounts for the high
75 recurrence rate of up to 50%, and the progression from milder forms to the more aggressive
76 types (Knottenbelt, 2005; Martens et al., 2001b; Marti et al., 1993).

77 BPV alters the expression of several genes in transformed fibroblasts. Upregulation of
78 the chemokine CXCL5 indicates an inflammatory response which is assumed to facilitate
79 sarcoid growth/invasion. Concurrent downregulation of immune response genes, such as
80 TLR-4 and MHC-I, likely enables viral infection and persistence (Yan et al., 2008a; Yuan et
81 al., 2010a). Interestingly, matrix metalloproteinase-1 (MMP-1) and -9 are also markedly

upregulated. MMPs may be responsible for tumour invasiveness since their expression and BPV-1 infection supports fibroblast invasion *in vitro* (Yan et al., 2008a; Yuan et al., 2010b).

We aimed to assess local tumour behaviour and *in vivo* MMP expression in naturally occurring sarcoids using histology, immunohistological detection of MMP-1, -2 and -9, and transmission electron microscopy (TEM).

Materials and methods

Animals and tissues

Forty-three naturally occurring surgically excised sarcoids from 25 horses (13 geldings, each 4 stallions and mares, 4 gender unknown) of various breeds and ages (2-21 years; average: 9.6 years) were studied. The tumours derived from various anatomic sites including the face, abdominal wall, axilla and medial thigh. None had been subjected to previous treatment. Individual lesions were classified clinically (Knottenbelt, 2005) and were histologically confirmed.

Immediately after surgical excision representative samples were fixed in 10% non-buffered formalin for 1-3 days and routinely paraffin wax embedded. From selected specimens, a portion was trimmed into 1-2 mm thick slices and fixed in 4% paraformaldehyde (pH 7.4) with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4°C for TEM.

Control skin samples from two horses euthanised for reasons other than skin disease, were similarly prepared.

Histology and immunohistology

Consecutive sections (3-5µm) were prepared from paraffin wax embedded specimens and stained with haematoxylin and eosin (HE), Gomori Trichrome stain and Periodic Acid Schiff (PAS) reaction to highlight collagen and the epidermal basement membrane, respectively.

Immunohistology was used to demonstrate MMP-1, -2 and -9 and, in selected cases, cytokeratins (to highlight any potential tumour cell invasion into the epidermis), using cross reacting antibodies: rabbit anti-human MMP-1 (Ab-6), mouse anti-human APMA-activated native MMP-2 (clone A-Gel VC2), and mouse anti-human native MMP-9 (clone IIA5) (Thermo Fischer Scientific Anatomic Pathology, Runcorn, UK), all reacting with both the inactive and the active form of the enzymes, and mouse anti-human pan-cytokeratin (clone AE1/AE3; Dako, Glostrup, Denmark). The peroxidase anti-peroxidase method with antigen retrieval in heated citrate buffer (pH 6.0; MMP-1, MMP-9) or protease (cytokeratin) and the horseradish peroxidase method (Envision; Dako) without antigen retrieval (MMP-2) were applied (Kipar et al., 1995; Kipar et al., 2005). . Antibody binding was visualised with diaminobenzidine as chromogen, followed by Papanicolaou's haematoxylin counterstain.

Consecutive sections incubated with TBS-Tween and/or a non-reacting antibody against Feline Coronavirus and/or *Toxoplasma gondii* instead of the primary antibody served as negative controls.

Constitutive MMP expression in the normal skin was assessed on the two control skin specimens stained for the MMPs. A biopsy from an inflamed skin wound served as positive control for the markers.

Grading and statistical analysis

For MMP-1, the staining intensity of the neoplastic cells was scored over a scale from faint [(+); 0.5] to strong [+++; 3]. Where staining intensity varied between tumour areas, an

average was calculated from the range of scores (Table 1). The association between MMP-1 expression intensity and sarcoid clinical type was analysed using Kruskal-Wallis since the data were not normally distributed and there was no appropriate transformation. Mann-Whitney test was used to compare the sarcoid types pair-wise and the associations of MMP-1 expression with histopathological findings (picket fence formation (PFF), EH, RPF). A Bonferroni correction was applied post-hoc so results for the three pairwise compared groups are at 0.167 level of significance. The two types only existing as single samples (mixed and occult) were excluded from the analysis. Stata 13 (StataCorp) software was used for statistical analyses.

Transmission electron microscopy

Local tumour cell behaviour was assessed by TEM on case 14b with epidermal RPF, cases 12 and 16 with PFF and case 13b with dermal infiltrative growth. Tissue blocks (2 mm³) were prepared from the fixed specimens, post-fixed in 1% osmium tetroxide_(aq) and routinely resin embedded. Toluidine blue stained semi-thin sections (0.75µm) identified areas of interest from which ultrathin sections (80 nm) were subsequently prepared and examined.

Results

Clinical information, classification and histological features of sarcoids

Sarcoids were clinically classified as nodular (n=19), verrucose (n=11), fibroblastic (n=11), occult (n=1) and mixed (n=1) (Table 1). They presented typical histological features and were variably cellular, non-demarcated aggregates of spindle shaped fibroblastoid cells in an irregular collagenous stroma (Martens et al., 2000; Scott and Miller, 2003).

In 37 cases, intact epidermis at least partly covered the neoplastic infiltrate. It exhibited epidermal hyperplasia (EH; n=24), RPF (n=20), and/or PFF (n=32), and appeared unaltered in only 3 nodular sarcoids. PFF was always present in the verrucose and fibroblastic sarcoids (Table 1A, B). In verrucose sarcoids, RPF occurred only alongside EH (2/6). Nodular sarcoids always showed EH together with both RPF and PFF, but either both (2/6) or none (4/6) of the latter without EH (Table 1).

Expression of MMP-1, -2 and -9 in the skin and in sarcoids

The normal skin showed intense constitutive MMP-1 expression, represented by a diffuse cytoplasmic reaction in epithelial cells of epidermis, hair follicles and adnexal glands, in vascular and arrector pili smooth muscle cells, and in endothelial cells (Fig. S1A). In contrast, MMP-2 and MMP-9 expression was very weak and limited to epithelial cells (Fig. S1B, C). Similar MMP expression was generally found in the skin over and around sarcoid (Fig. 1A). However, epithelial MMP-2 expression was variable, often lacking (n=22) and sometimes (n=6) slightly more intense than in the control skin.

In all tumours, neoplastic cells expressed MMP-1, evident as a diffuse cytoplasmic staining (Fig. 1A). Staining intensities varied, both between specimens and within individual tumours. Comparison of the overall staining intensity in the larger tumour cohorts showed the highest mean expression in nodular (2.158) and the lowest in verrucose sarcoids (1.545), fibroblastic sarcoids showed only a slightly higher average (1.659). Since the MMP-1 expression intensity measurements were not normally distributed, the differences were examined using non-parametric tests rather than comparison of the means. Kruskal Wallis test identified significant differences between the different sarcoid types (Kruskal-Wallis: $\chi^2_{d.f.:2} = 6.288$, $p < 0.05$) while pair-wise testing using Mann-Whitney identified differences between the verrucose and nodular sarcoids (Mann-Whitney $z = -2.581$, $p < 0.05$).

In many cases, there was evidence of an expression gradient in association with PFF (most intense close to epidermis; Fig. 1B), (focal) ulceration (strongest reaction in proximity to ulceration) and granulation tissue formation (strongest reaction in neoplastic cells intermingled with new vessels; Fig. S2A, B) though this spatial association was not reflected in the association between the overall presence of PFF and the intensity of MMP-1 expression. In some cases, a weak reaction was also seen in pre-existing fibrocytes and nerve fibres in the tumour-free dermis, and in the serum.

Univariable non-parametric analysis using Mann-Whitney test identified no significant association between the intensity of MMP-1 expression and the presence of EH (Mann-Whitney $z=0.247$, $p>0.05$), RPF (Mann-Whitney $z=-1.723$, $p>0.05$) and PFF (Mann-Whitney $z=1.707$, $p>0.05$). Because of the lack of normal distribution the analysis does not consider the effect that the different histopathological features may have on the expression of the MMP-1 or the clustering effect of the origin of some sarcoids from the same animal. Multivariable analysis on a larger sample could help clarify such associations.

Neoplastic cells were generally MMP-2 negative. In 10 cases (occult, 2 nodular, 3 verrucose, 4 fibroblastic sarcoids), however, they exhibited very weak MMP-9 expression (Fig. 1C).

Many cases (17/38 with natural surface; Table 1), and in particular the fibroblastic (9/10) and nodular (5/15) types exhibited variably extensive ulceration. In 4 tumours, this was chronic, with marked granulation tissue formation (Fig. S2A). Occasionally, mild peripheral perivascular mononuclear infiltration was also seen (Fig. S2A). Macrophages within infiltrates expressed MMP-1 (Fig. S2B), MMP-2 (Fig. S2C) and, occasionally, MMP-9. In areas of early ulceration, keratinocytes were strongly MMP-2 positive (Fig. S2D).

Local infiltrative growth and interaction between neoplastic cells and epidermis in sarcoids

Tumour infiltrates were not demarcated. Instead, neoplastic fibroblasts infiltrated the pre-existing dermal collagenous matrix (Fig. 2A). Within the tumour mass, collagen bundles were irregular and less densely packed than in the normal dermis (Fig. 2B) and stained positively for MMP-1 (Fig. 2C). TEM indicated that they were loosened around neoplastic cells (Fig. 2D).

Interaction between epidermis and neoplastic cells was indicated by PFF, the perpendicular alignment of tumour cells along the epidermis (Fig. 3A) without infiltration of the latter, as confirmed by immunohistology for pan-cytokeratin. PFF was identified in 86% of cases (32/37) with intact epidermis and in all clinical types (Table 1). In PFF areas, the dermo-epidermal junction was often obscured, as neoplastic cells and keratinocytes exhibited equally strong MMP-1 expression (Fig. 3B). The basement membrane (BM) appeared thickened and/or loosened in the PAS reaction, and in semi-thin sections, interdigitation of neoplastic cells and keratinocytes in the basal epidermal layer became obvious (Fig. 3C). TEM showed that the BM was absent in these areas (Fig. 3D). In contrast, the BM was continuous when PFF was not observed (Fig. 3E, F). In all cases, the dermal collagen appeared disintegrated where neoplastic cells were present (Fig. 3D, F).

Discussion

Equine sarcoids originate from fibroblasts transformed by BPV that acquire the capacity to invade the extracellular matrix (ECM) through the expression of MMPs (Yuan et al., 2008b; Yuan et al., 2010b; Hartl et al., 2011). In the present *in vivo* study, histology, immunohistology for MMP-1, -2 and -9, and TEM were used to assess local behaviour and MMP expression patterns in sarcoids.

Our study confirms that histology does not allow sub-classification, since all relevant morphological features are observed in all clinical types (Pascoe and Knottenbelt, 1999; Martens et al., 2000; Knottenbelt, 2005). Hyperplasia and rete peg formation of the overlying epidermis (EH, RPF) and interaction of neoplastic cells with the epidermis (PFF) are also typically found in all types, to varying extent.

Sarcoids are locally aggressive, recur frequently and can progress rapidly from clinically benign to more aggressive forms (Tarwid et al., 1985; Marti et al., 1993; Martens et al., 2001b; Knottenbelt, 2005). This is reflected histologically by local infiltrative growth of the neoplastic cells (Martens et al., 2000) which we found in association with apparent loosening of the dermal collagen matrix. We observed strong MMP-1 expression by neoplastic cells and MMP-1 deposition in the ECM; this suggests that MMP-1 contributes to the ECM changes.

MMP-1 is an interstitial collagenase and is expressed by several cell types, including fibroblasts, keratinocytes and macrophages; it predominantly degrades collagen III. Expression is regulated at transcriptional level, and induced, for example, by cytokines and growth factors (Shapiro, 1998; Westermarck and Kähäri, 1999; Fields, 2013). Produced in an inactive form, MMP-1 is activated by several enzymes (Kähäri and Saarialho-Kere, 1997; Westermarck and Kähäri, 1999). In equine sarcoids, MMP-1 is present in its active form (Yuan et al., 2010b), and its release by neoplastic cells may be responsible for infiltrative dermal tumour growth, via partial destruction of the ECM, i.e. the cleaving of collagen III. This indicates increased ECM turnover, and indeed, sarcoids have been shown to exhibit an overall lower amount of collagen than the normal equine dermis, though an increased and more organised, fibrous collagen III network (Williams et al., 1982). The fate of the cleaved collagen III remains unclear, since further degrading requires the gelatinases, MMP-2 and -9

(Shapiro, 1998; Visse and Nagase, 2003; Fields, 2013), neither of which were found to be upregulated in the present nor a previous study (Yuan et al., 2010b).

We demonstrated MMP-1 expression in sarcoids by both neoplastic fibroblasts and epithelial cells, in particular epidermal keratinocytes, and with similar intensity. Assuming that the active form is present, MMP-1, which can degrade several BM components, could also be responsible for PFF in sarcoids since it accompanies loss of BM continuity. In support of this an *in vitro* study showed that sarcoid fibroblast-derived cells invaded a 3D Matrigel model composed of several BM components (Yuan et al., 2010b).

The epidermal BM is a thin (<100 nm), but vital interface between basal keratinocytes and dermis. Its upper lamina lucida and lower lamina densa solidly attach the epidermis to the dermis. This anchoring system is composed of hemidesmosomes at the basal pole of the basal keratinocytes, thin anchoring filaments that traverse the lamina lucida, and anchoring fibrils (composed of collagen VII loops) originating from the lamina densa and extending towards the dermis, as well as collagen IV and laminin networks which provide the basic scaffolding of the BM and are linked via nidogen. The keratin filament of the hemidesmosomes binds to laminin 5 which in turn binds to, among others, collagen VII (McMillan et al., 2003; Breitkreutz et al., 2013). MMP-1 can degrade laminins and collagen VII; with the destruction of these two proteins, i.e. the anchoring fibrils and one of the two main networks (Shapiro, 1998; Villone et al., 2008), the BM would lose its normal stable architecture. This could account for the BM destruction seen with PFF in sarcoids and might also render the skin overlying the neoplastic infiltrate more vulnerable and liable to ulceration.

The local behaviour of sarcoids differs from malignant tumours that invade the epidermis, such as human cutaneous melanomas in which MMP-2 and -9 are expressed by neoplastic cells and degrade collagen IV, thereby destroying the BM scaffolding, (Väisänen et al., 1996; Chen et al., 2012; Breitkreutz et al., 2013) (). This is not encountered in sarcoids.

However, MMP-2 is also expressed by hyperproliferative keratinocytes (Krengel et al., 2002) which are found in the epidermis overlying equine sarcoids (Martens et al., 2000). This may account for the slight increase in epidermal MMP-2 expression in some of our cases. The latter was particularly obvious in association with PFF, suggesting that direct contact between epidermis and fibroblasts is a prerequisite of proliferation (Martens et al., 2000). Alternatively, BPV-1, found to productively infect the epidermis overlying natural sarcoids (Brandt et al., 2011), could induce keratinocyte hyperproliferation.

In contrast, the strong MMP-2 expression that we observed in keratinocytes in early ulceration could result from cytokine and MMP-1 release by inflammatory cells (Kähäri, 1997). Similarly, the pronounced MMP-1 expression of neoplastic cells might result from local cytokine release with ulceration, granulation tissue formation and PFF (Westermarck and Kähäri, 1999).

Our observation that normal equine epidermis and follicular and glandular epithelium constitutively express MMP-1 differs from a previous study (Miragliotta et al., 2008). This could be due to differences in the detection sensitivity since different antibodies and detection systems were used.

The equine sarcoid remains a clinical challenge and there remains a high risk of failure with all treatment methods (Knottenbelt and Kelly, 2000). Invasion is the major constraint to therapy. The likelihood of recurrence is highest with surgical methods that do not include a safe margin but the difficulty in defining a safe margin for any individual lesion is a significant surgical challenge. The likelihood of recurrence is seemingly correlated with the presence of BPV DNA in the surgical margins (Martens et al., 2001c). Topical and intralesional chemotherapy options may fail because the tumour extends beyond the reach of the chemotherapeutic agent (Knottenbelt and Kelly, 2000). This study suggests that, excluding the malignant form which we have not examined, the risks of invasion are not

significantly different in any particular sarcoid subtype. Early, pre-emptive, use of medications that could alter the tendency to invasion through manipulation of MMP-1 expression might provide improved outcomes when they are subsequently treated either surgically or medically.

Conclusions

The present *in situ* study demonstrates a correlation between the expression of MMP-1 by neoplastic and epithelial cells and the local behaviour of sarcoids. The release of MMP-1 could be responsible for the consistently dermal invasion of the neoplastic cells, through the degradation of collagen III, and could, through partial destruction of the BM, mediate PFF. These results which were obtained in all clinical types of sarcoids suggest potential new approaches for the therapy of sarcoids.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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462 **Table legends**

463 **Table 1.**

464 **A.** Sarcoid cases with clinical type, relevant histological features and intensity of MMP-1
465 expression.

466 **B.** Summary results of the examination for the presence of relevant histopathological features
467 of the epidermis and its interaction with the neoplastic cells in the different sarcoid types.

468

469

Figure legends

Fig. 1. MMP-1 and -9 expression in neoplastic cells. PAP method. A. Nodular sarcoid (case 14c) with epidermal hyperplasia. The epidermis (E) shows moderate, the neoplastic cells (arrows) weak MMP-1 expression. Activated endothelial cells in a vein are also strongly MMP-1 positive (arrowhead). Bar = 20µm. B. Nodular sarcoid with picket fence formation (case 9b). MMP-1 is strongly expressed by keratinocytes in the epidermis (E). Neoplastic cells are diffusely positive, but show the strongest expression intensity close to the epidermis (arrow). Bar = 50µm. C. Fibroblastic sarcoid (case 23), exhibiting weak MMP-9 expression by neoplastic cells (arrows). Bar = 10µm.

Fig. 2. Dermal infiltrative growth. A. Nodular sarcoid (case 11) with picket fence formation. In the dermis, the neoplastic infiltrate is not demarcated and neoplastic fibroblastoid cells infiltrate into the adjacent pre-existing collagen rich extracellular matrix (arrows). HE stain. Bar = 50µm. B. Left: Nodular sarcoid (case 14c). The cells are embedded in loosely arranged thin collagen strands. Right: Normal dermis, comprised of densely packed wavy collagen bundles with a few embedded unaltered fibrocytes. Gomori Trichrome stain. Bars = 10µm. C. Nodular sarcoid (case 4b) with intense MMP-1 expression in neoplastic cells (arrows). There is also a moderate reaction in the extracellular matrix (arrowhead). PAP method. Bar = 10µm. D. Nodular sarcoid (case 13b). Transmission electron micrograph of the dermal neoplastic infiltrate. Several tumour cells (arrows) with prominent cell projections, infiltrating the pre-existing collagen rich extracellular matrix that contains dense bundles of short collagen fibres (asterisks) which appear loosened around the neoplastic cells. Bar = 5µm.

Fig. 3. Interaction between epidermis and neoplastic cells. A. Fibroblastic sarcoid (case 7) with picket fence formation (PFF), represented by the perpendicular arrangement and interdigitation of the subepithelial neoplastic cells with the basal cells of the epidermis (arrows). HE stain. Bar = 20µm. B. Verrucose sarcoid with PFF (case 8a) with strong MMP-1 expression of both epidermis and neoplastic cells, obscuring the dermo-epidermal junction (arrows). PAP method. Bar = 20µm. C. Nodular sarcoid with PFF (case 16). The epidermal basement membrane (BM) appears multifocally lost due to the interdigitation of neoplastic cells with the basal cells of the epidermis (arrows). Semithin section, Toluidine blue stain. Bar = 10µm. D. Fibroblastic sarcoid with PFF (case 12). Transmission electron micrograph (TEM). The basal epithelial cells (BE) of the epidermis are devoid of the BM and show loss of cell-cell contact (acantholysis; arrows). The subepithelial collagen (asterisks) is disintegrated and closely apposed to a tumor cell (TC) with its long cell projections. Bar = 2µm. E, F. Nodular sarcoid (case 14b) with rete peg formation, but no PFF. E. Neoplastic cells are positioned close to the BM, but do not interdigitate with the epidermis (arrows). Semithin section, Toluidine blue stain. Bar = 10µm. F. TEM. The basal epithelial cells (BE) of the epidermis exhibit a well defined continuous BM (arrows). The subepithelial collagen (asterisks) is disintegrated and closely apposed to tumour cells (TC). Bar = 2µm.

513 Supplementary material (Figures S1 and S2): Figure legends:

514 Fig. S1. Expression of MMP-1, -2 and -9 in the normal equine skin (control case). A. MMP-1
515 is moderately to strongly expressed by epithelial cells in epidermis (E), hair follicles (HF)
516 and sebaceous (SG) and apocrine glands (arrows). In addition, smooth muscle and endothelial
517 cells in blood vessels (V) as well as the smooth muscle cells of the arrector pili muscles
518 (arrowheads) show a positive reaction. PAP method. Bar = 50µm. B. MMP-2 expression is
519 generally very weak and restricted to epithelial cells in epidermis (E), hair follicles (HF) and
520 sebaceous (SG) and apocrine glands (arrows). HRP method. Bar = 50µm. C. MMP-9
521 expression is generally weak and seen in epithelial cells in epidermis (E), hair follicles (HF)
522 and sebaceous (SG) and apocrine glands (arrows). PAP method. Bar = 50µm.

523

524 Fig. S2. Ulceration and granulation tissue formation in sarcoids. A. Fibroblastic sarcoid (case
525 5) with chronic ulceration, with underlying granulation tissue with numerous new vessels
526 (arrowheads). Inset: Occult sarcoid (case 24) with focal mononuclear perivascular infiltration.
527 Bar = 50µm (inset: Bar = 20µm). HE stain. B. Mixed sarcoid (case 3) with extensive
528 ulceration and granulation tissue formation. MMP-1 is expressed by inflammatory cells and
529 proliferating fibroblasts in the granulation tissue (arrows) and by endothelial cells forming the
530 new vessels (arrowheads). Neoplastic cells also exhibit moderate MMP-1 expression. Inset:
531 Occult sarcoid (case 24). Leukocytes in the mononuclear perivascular infiltrate exhibit
532 moderate MMP-1 expression. PAP method. Bar = 50µm (inset: Bar = 20µm). C. Fibroblastic
533 sarcoid (case 5) with ulceration and granulation tissue formation. MMP-2 expression is seen
534 in infiltrating macrophages in the inflammatory process. Endothelial cells in new vessels
535 (arrowheads) and neoplastic cells are MMP-2 negative. HRP method. Bar = 50µm. D.
536 Verrucose sarcoid (case 6) with early ulceration, exhibiting strong MMP-2 expression by
537 epithelial cells (arrowheads). HRP method. Bar = 50µm.